Supplemental Material

Supplemental Material, Figure 1

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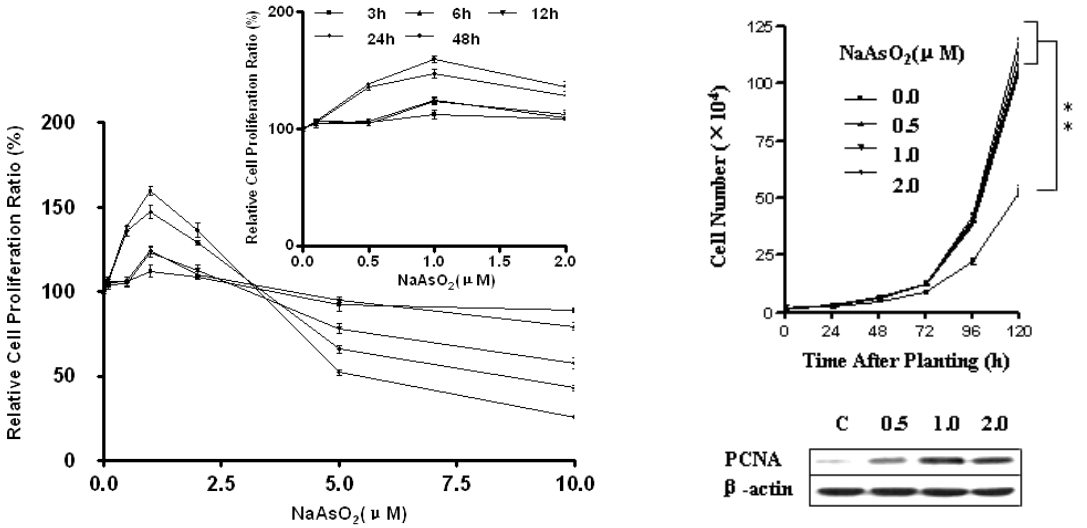

Figure 1. The effects of arsenite on growth of HELF cells. (A) Proliferation efficiency of HELF cells exposed to different levels of arsenite for different times (means ± SD, n = 3). After HELF cells were exposed to 0.0, 0.1, 0.5, 1.0, 2.0, 5.0, or 10.0 μM arsenite for 3, 6, 12, 24, or 48 hr, their proliferation was measured by use of a cell counting kit-8 assay. The relative ratios of cell proliferation were determined by comparing growth of cells exposed to no arsenite. (B) Growth curves (top) and levels of PCNA (bottom) in passage control and arsenite-transformed HELF cells (means ± SD, n = 3). After HELF cells were exposed to 0.0, 0.5, 1.0, or 2.0 μM arsenite for about 15 weeks (30 passages), their doubling times were calculated, and the levels of PCNA were determined by Western blots. β-Actin levels, measured in parallel, served to standardize the values. $^{**}P < 0.01$ difference from passage control cells.

Supplemental Material, Figure 2

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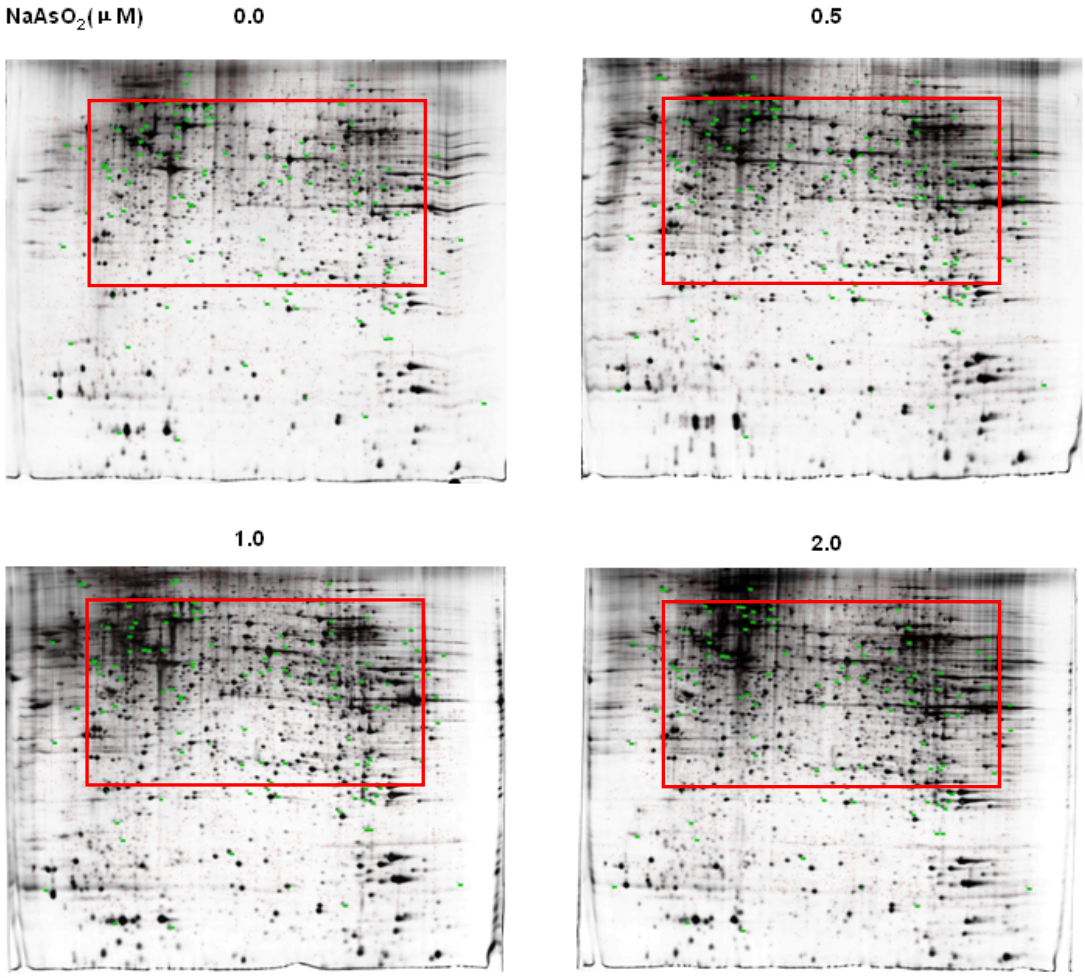

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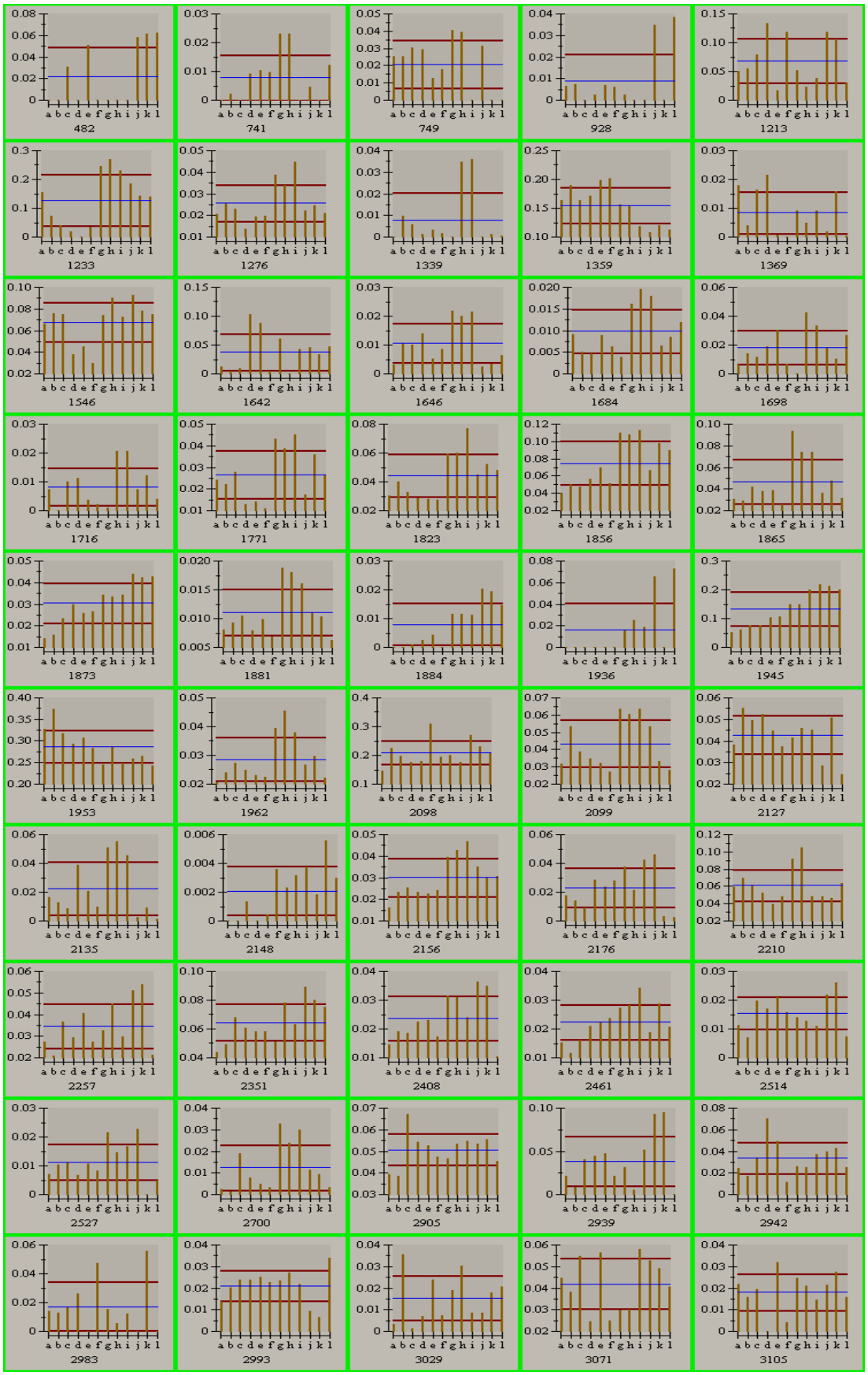
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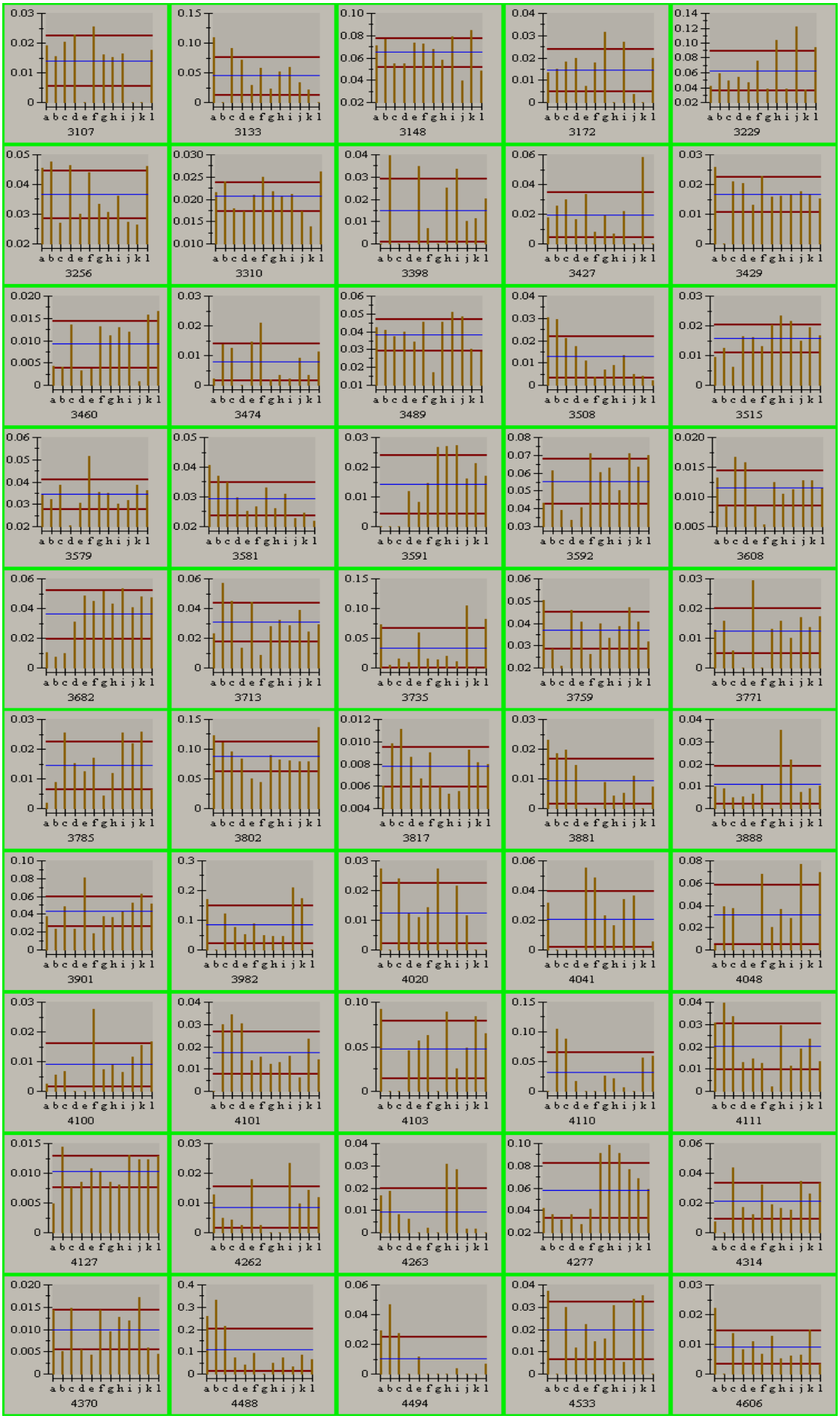
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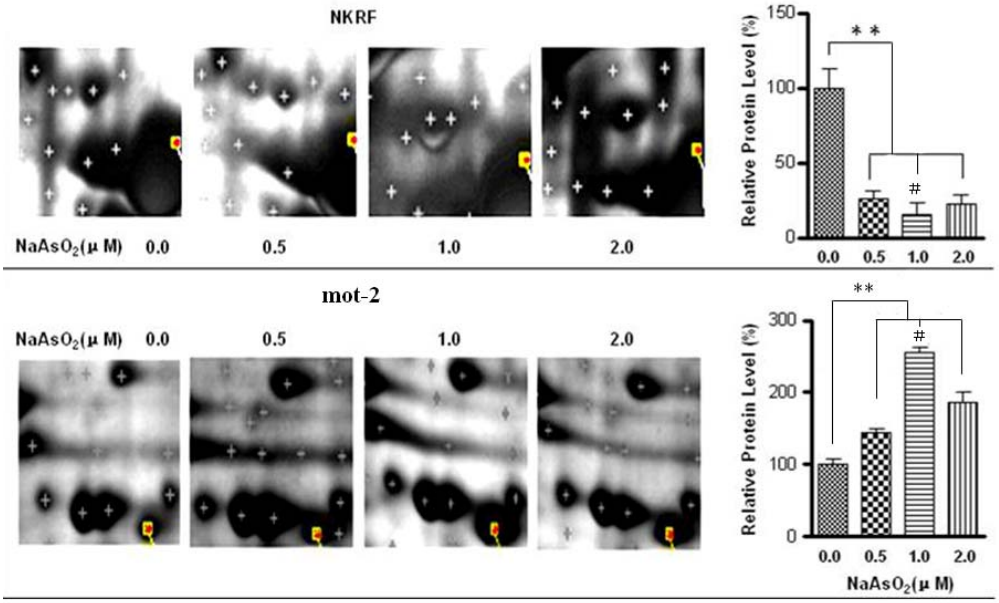

Figure 2. Alterations of cellular protein levels induced by low levels of arsenite in HELF cells. After HELF cells were exposed to 0.0, 0.5, 1.0, or 2.0 μM arsenite for about 15 weeks (30 passages), cellular proteins were detected and analyzed by 2-dimensional electrophoresis (2DE). (A) Images of 2DE-gels. A total of 126 protein spots were different between passage control cells and cells transformed by arsenite; most of these are present in the red frame. (B) Differences analyzed by ImageMaster^{TM} two-dimensional Platinum software (n = 3). The ordinates represent relative volume (% volume), which normalizes the spot volume as a percentage of the total volume of all spots present in a gel. a,b,c: no arsenite; d,e,f: 0.5 μM arsenite; g,h,i : 1.0 μM arsenite; j,k,l: 2.0 μM arsenite. (C) The 2DE mages (left) and the levels (right) of NKRF and mot-2 (means ± SD, n = 3). In arsenite-transformed cells, there was down-expression of NKRF and up-expression of mot-2 (**$p < 0.01$ difference from passage control cells). The changes in cells exposed to 1.0 μM arsenite were more marked than that for cells exposed to 0.5 μM or 2.0 μM arsenite, ($^{\#}p < 0.05$, difference from cells exposed to 0.5 μM and 2.0 μM arsenite).

Supplemental Material, Figure 3

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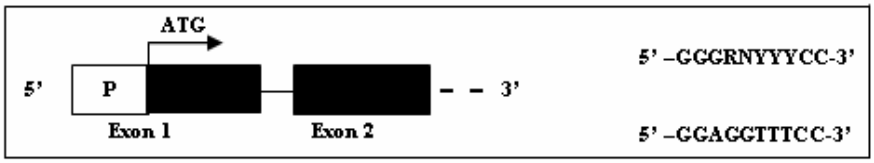

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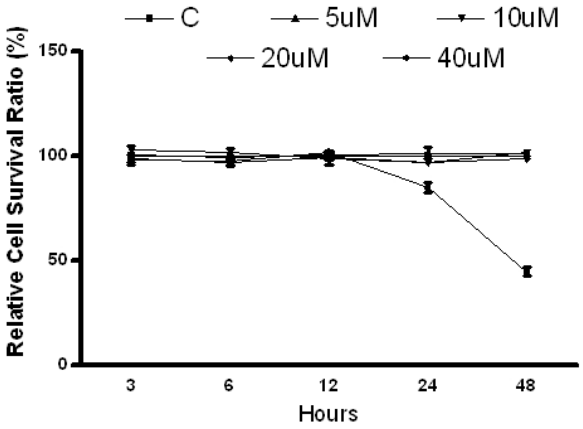

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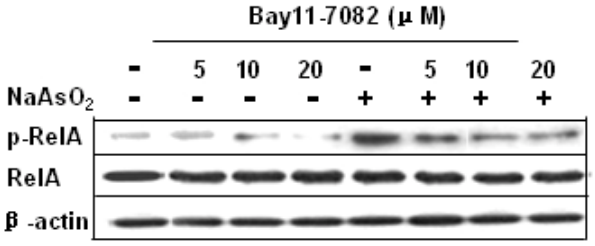

Figure 3. Schematic representation of the mot-2 gene and the concentration of Bay11-7082. (A) Schematic representation of the *mot-2* gene and its promoters. The sequence *GGAGGTTTCC* of the *mot-2* promoter is similar to kappaB DNA elements (*GGGRNYYYCC*). (R is an unspecified purine, Y is an unspecified pyrimidine, and N is any nucleotide.) (B) The effects of Bay11-7082, an NF-κB inhibitor, on proliferation of HELF cells (means ± SD, n = 3). After cells were exposed to 0.0, 5.0, 10.0, 20.0, or 40.0 μM Bay11-7082 for 3, 6, 12, 24, or 48 hr, cell proliferation was

measured by use of a cell counting kit-8 assay. The relative ratios of cell survival were plotted with untreated cells determining the 100% activity level. (C) Bay11-7082 blocking the activation of NF-κB induced by arsenite. After cells were exposed to 0.0, 5.0, 10.0, or 20.0 μM Bay11-7082 for 3 hr, cells were exposed to 0.0 or 1.0 μM arsenite for 3 hr. Cell lysates were subjected to Western blots with RelA, p-RelA, and β-actin antibodies. β-Actin levels, measured in parallel, served to standardize the values.

Supplemental Material, Figure 4

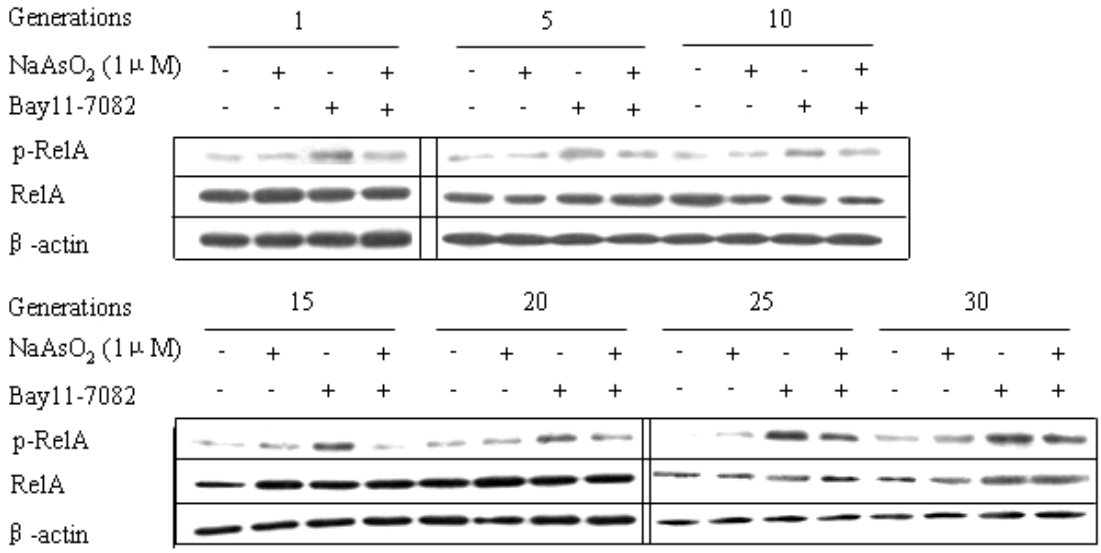

Figure 4. The effects of Bay11-7082 on NF-κB activation during arsenite-induced neoplastic transformation of HELF cells. After cells were incubated with 0.0 or 1.0 μM arsenite for 0, 5, 10, 15, 20, 25, or 30 passages or to arsenite in combination with 10.0 μM Bay11-7082, cell lysates were subjected to Western blots with RelA, p-RelA, and β-actin antibodies. β-Actin levels, measured in parallel, allowed standardization of the values. In cultures exposed to 1.0 μM arsenite, there were more malignant cells and greater expression of p-RelA, effects blocked by Bay11-7082.

Supplemental Material, Figure 5

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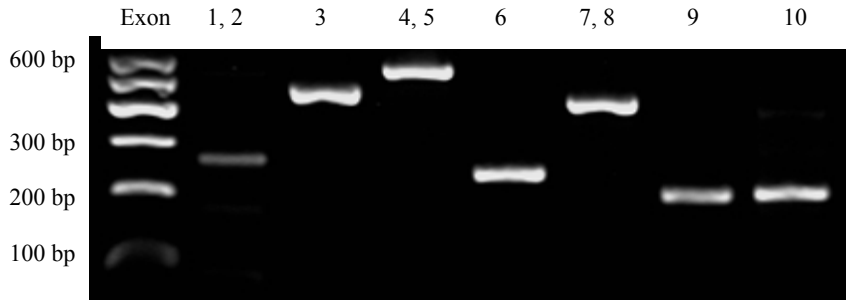

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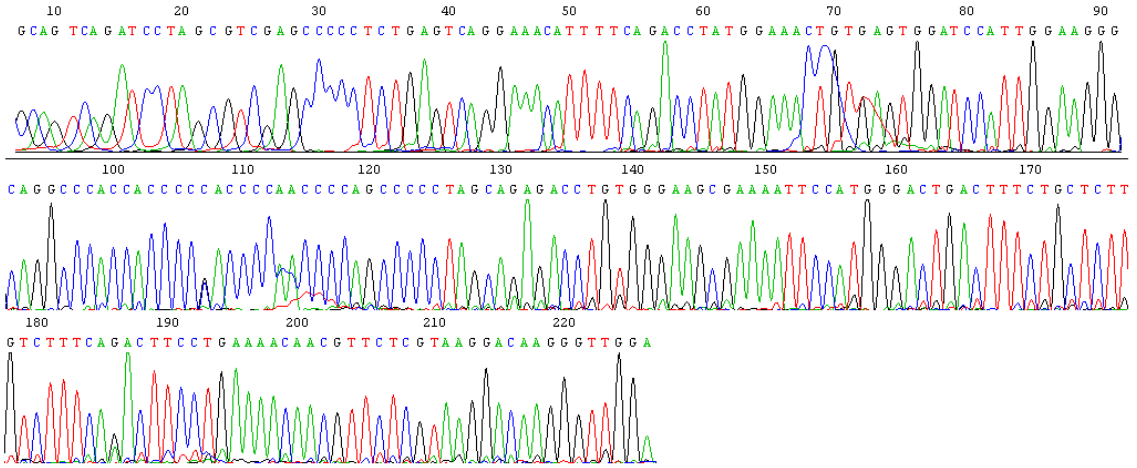

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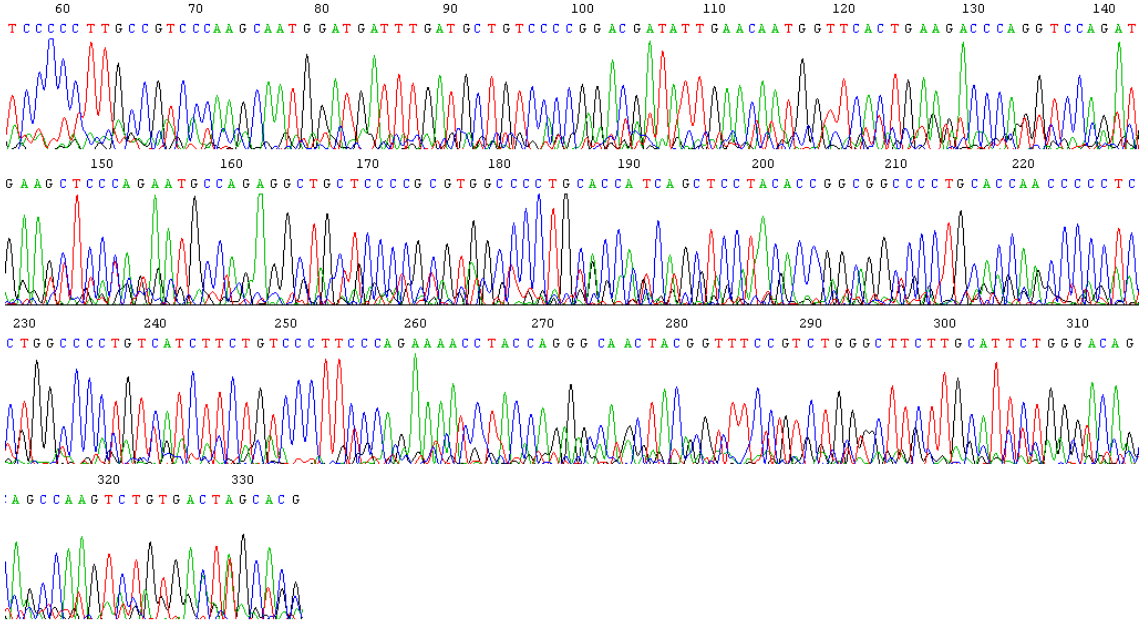

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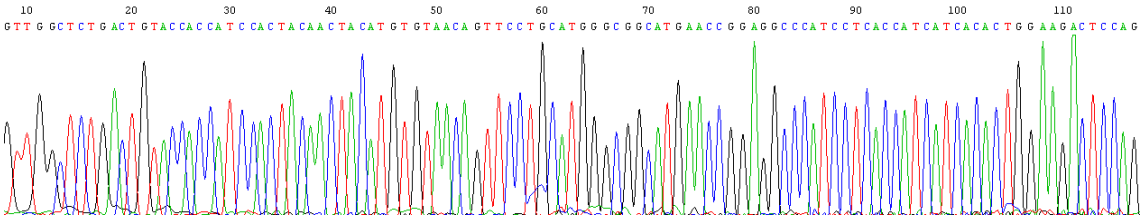

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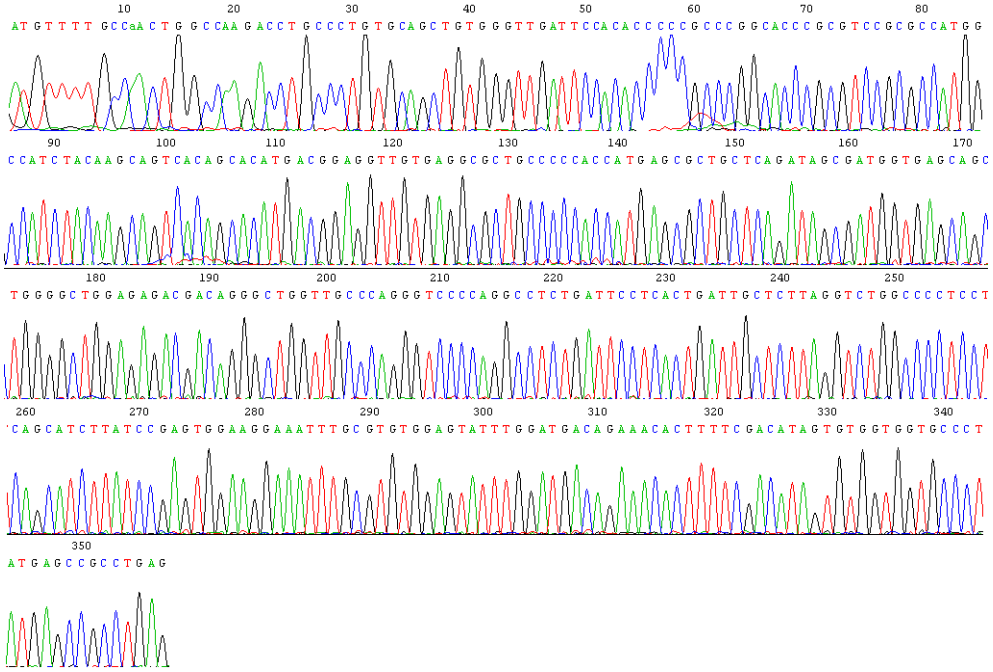

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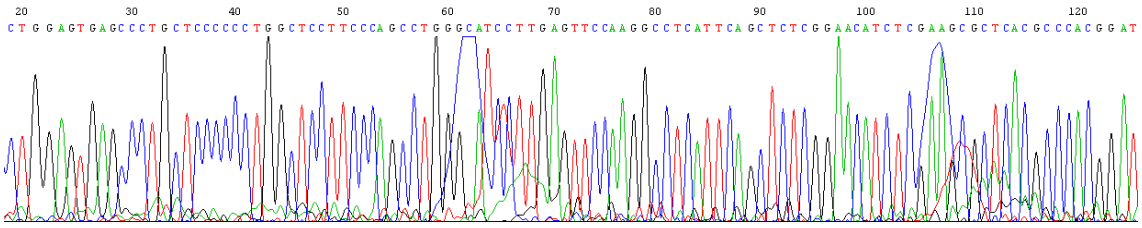

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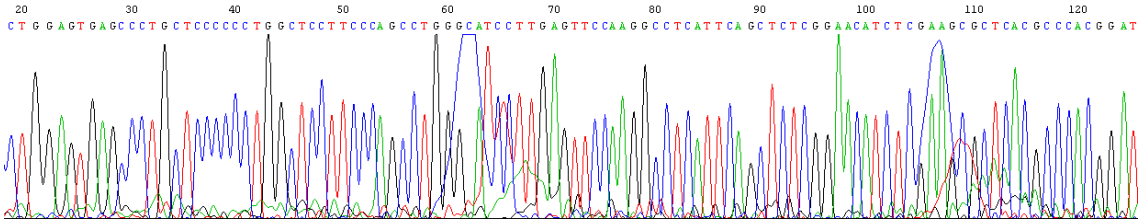

Figure 5. Lack of mutations in p53 cDNA in arsenite-induced transformed cells. (A) DNA fragments, including exons 1-10 of *p53*, were amplified by PCR. Primers used for amplification were as following: Exon 1,2: *5'-CAGCAGCCAGACTGACCTACG-3', 5'-CCAACCCTTGTCCTTACGAGAA-3';* exon 3: *5'-TCTGGTAAGGACAAGGGT-3', 5'-TCCAAACAAAAGAAATGC-3';* exon 4,5: *5'-CTCTATCTCCTTCCTCTT-3', 5'-TTTACTTTGGACATCTCA-3'*; exon 6: *5'-CCACAGGTCTCACCAAGG-3', 5'-AGAGGTCAGAGGCAAGCA-3'*; exon 7,8: *5'-GGACCTGATTTCCATAGTG-3', 5'-ACGGCATTTTGAGTGTTA-3'*; exon 9: *5'-CTTCTCCCCCTCCTCTGT-3', 5'-CCTATGGCTTTCCAACCT-3'*; and exon 10: *5'-GGTCAGGGAAAAGGGGCA-3', 5'-AGATGGGGGTGGGAGGC-3'*

(B) DNA fragments including exons 1-10 of *p53* were sequencing using primers of PCR amplification. As determined by blast analysis, the DNA sequences of exons 1-10 were a 100% match to *p53* cDNA, indicating that there was no mutation in *p53* cDNA in cells with arsenite-induced transformation. a: exon 1, 2; b: exon 3; c: exon 4, 5; d: exon 6; e: exon 7, 8; f: exon 9; g: exon 10.